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&AF/1645

Practitioner's Docket No. MSU 4.1-542 **PATENT**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Alberto L. Mendoza
Application No.: 09/998,822 Group No.: 1645
Filed: November 1, 2001 Examiner: Nita M. Minnifield
For: VACCINE FOR PREVENTING PYTHIOSIS IN HUMANS AND ANIMALS

Mail Stop Appeal Briefs – Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

TRANSMITTAL OF APPEAL BRIEF
(PATENT APPLICATION—37 C.F.R. § 1.192)

NOTE: The phrase "the date on which" an "appeal was taken" in 35 U.S.C. 154(b)(1)(A)(ii) (which provides an adjustment of patent term if there is a delay on the part of the Office to respond within 4 months after an "appeal was taken") means the date on which an appeal brief under § 1.192 (and not a notice of appeal) was filed. Compliance with § 1.192 requires that: 1. the appeal brief fee (§ 1.17(c)) be paid (§ 1.192(a)); and 2.the appeal brief complies with § 1.192(c)(1) through (c)(9). See Notice of September 18, 2000, 65 Fed. Reg. 56366, 56385-56387 (Comment 38).

1. Transmitted herewith, in triplicate, is the APPEAL BRIEF in this application, with respect to the Notice of Appeal filed on 03/26/04.

NOTE: "Appellant must, within two months from the date of the notice of appeal under § 1.191 or within the time allowed for reply to the action from which the appeal was taken, if such time is later, file a brief in triplicate. . . " 37 C.F.R. § 1.192(a) (emphasis added).

CERTIFICATION UNDER 37 C.F.R. §§ 1.8(a) and 1.10*
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Jessica R House
Signature

Date: 04/23/04

Jessica R. House
(type or print name of person certifying)

** Only the date of filing (§ 1.6) will be the date used in a patent term adjustment calculation, although the date on any certificate of mailing or transmission under § 1.8 continues to be taken into account in determining timeliness. See § 1.703(f). Consider "Express Mail Post Office to Addressee" (§ 1.10) or facsimile transmission (§ 1.6(d)) for the reply to be accorded the earliest possible filing date for patent term adjustment calculations.*

2. STATUS OF APPLICANT

This application is on behalf of

☐ other than a small entity.

☒ a small entity.

A statement:

☐ is attached.

☒ was already filed.

3. FEE FOR FILING APPEAL BRIEF

Pursuant to 37 C.F.R. § 1.17(c), the fee for filing the Appeal Brief is:

☒	small entity	$165.00
☐	other than a small entity	$330.00

Appeal Brief fee due $ 165.00

4. EXTENSION OF TERM

NOTE: 37 C.F.R. § 1.704(b) ". . .an applicant shall be deemed to have failed to engage in reasonable efforts to conclude processing or examination of an application for the cumulative total of any periods of time in excess of three months that are taken to reply to any notice or action by the Office making any rejection, objection, argument, or other request, measuring such three-month period from the date the notice or action was mailed or given to the applicant, in which case the period of adjustment set forth in § 1.703 shall be reduced by the number of days, if any, beginning on the day after the date that is three months after the date of mailing or transmission of the Office communication notifying the applicant of the rejection, objection, argument, or other request and ending on the date the reply was filed. The period, or shortened statutory period, for reply that is set in the Office action or notice has no effect on the three-month period set forth in this paragraph."

NOTE: The time periods set forth in 37 C.F.R. § 1.192(a) are subject to the provision of § 1.136 for patent applications. 37 C.F.R. § 1.191(d). See also Notice of November 5, 1985 (1060 O.G. 27).

NOTE: As the two-month period set in § 1.192(a) for filing an appeal brief is not subject to the six-month maximum period specified in 35 U.S.C. § 133, the period for filing an appeal brief may be extended up to seven months. 62 Fed. Reg. 53,131, at 53,156; 1203 O.G. 63, at 84 (Oct. 10, 1997).

The proceedings herein are for a patent application and the provisions of 37 C.F.R. § 1.136 apply.

(complete (a) or (b), as applicable)

(a) ☐ Applicant petitions for an extension of time under 37 C.F.R. § 1.136 (fees: 37 C.F.R. § 1.17(a)(1)-(5)) for the total number of months checked below:

Extension (months)	Fee for other than small entity	Fee for small entity
☐ one month	$ 110.00	$ 55.00
☐ two months	$ 420.00	$ 210.00
☐ three months	$ 950.00	$ 475.00
☐ four months	$ 1,480.00	$ 740.00
☐ five months	$ 2,010.00	$ 1,005.00

Fee: $_____

If an additional extension of time is required, please consider this a petition therefor.

(check and complete the next item, if applicable)

☐ An extension for _____ months has already been secured, and the fee paid therefor of $_____ is deducted from the total fee due for the total months of extension now requested.

Extension fee due with this request $_____

or

(b) ☒ Applicant believes that no extension of term is required. However, this conditional petition is being made to provide for the possibility that applicant has inadvertently overlooked the need for a petition and fee for extension of time.

5. TOTAL FEE DUE

The total fee due is:

Appeal brief fee $ 165.00

Extension fee (if any) $_____

TOTAL FEE DUE $ 165.00

6. FEE PAYMENT

☒ Attached is a ☒ check ☐ money order in the amount of $ 165.00

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☐ Charge any additional fees required by this paper or credit any overpayment in the manner authorized above.

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NOTE: If there is a fee deficiency and there is no authorization to charge an account, additional fees are necessary to cover the additional time consumed in making up the original deficiency. If the maximum six-month period has expired before the deficiency is noted and corrected, the application is held abandoned. In those instances where authorization to charge is included, processing delays are encountered in returning the papers to the PTO Finance Branch in order to apply these charges prior to action on the cases. Authorization to change the deposit account for any fee deficiency should be checked. See the Notice of April 7, 1986, *1065 O.G. 31-33.*

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SIGNATURE OF PRACTITIONER

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Alberto L. Mendoza

Serial No.: 09/998,822 Group Art Unit: 1645

Filed : November 1, 2001

For : VACCINE FOR PREVENTING PYTHIOSIS IN HUMANS AND ANIMALS

Examiner : Nita M. Minnifield

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BRIEF UNDER 37 CFR § 1.192

Sir:

This is an appeal from a final rejection in the above entitled application. The claims on appeal are set forth as Appendix A. An oral hearing will be requested. Enclosed are three (3) copies of this Brief and the fee due upon filing of the Brief.

(1) Real Party in Interest

The real party in interest is Michigan State University, East Lansing, Michigan, a constitutional corporation of the State of Michigan, which is the assignee of the above entitled application.

(2) Related Appeals and Interferences

There are no related appeals or interferences. This application is a continuation-in-part of U.S. application Serial No. 09/082,232, filed May 20, 1998, now U.S. Patent No. 6,287,573, which is a division of Serial No. 08/895,940, filed July 17, 1997, now U.S. Patent No. 5,948,413. This application also claims priority to provisional application Serial No. 60/245,936, filed November 3, 2000.

(3) Status of Claims

Claims 1-32 are pending in the application. Claims 1-3 and 13-32 have been withdrawn from consideration. Claims 4-12 have been rejected. No claims have been allowed.

(4) Status of Amendments

No amendments have been filed subsequent to final rejection.

(5) Summary of Invention

The invention in Claim 4 is a method for treatment of pythiosis or prophylaxis against pythiosis in a mammal which comprises: (a) providing an injectable vaccine which comprises in a sterile aqueous solution in admixture (Specification: page 13, lines 23-32): (i) intracellular cytoplasmic antigens separated from disrupted cells of *Pythium insidiosum* by SDS-PAGE (Specification: page 21, line 36 through page 22, line 8); and (ii)extracellular antigens secreted into a medium for growing the cells of the *Pythium insidiosum* wherein the mixture comprises 28, 30 and 32 kDa antigens as determined by SDS-PAGE (Specification: page 22, lines 4-11 and lines 32-34); and (b) vaccinating the mammal with the vaccine (Specification: page 22, line 35 through page 23, line 9; page 6, lines 8-9).

The invention in Claim 5 is the method of Claim 4 wherein the antigens have been provided by (a) growing cells of the *Pythium insidiosum* in a culture medium (Specification: page 20, lines 13-17; page 35,

lines 33-35) and then (i) killing the cells (Specification: page 20, line 18); (ii) separating the killed cells from the culture medium so as to produce a first supernatant comprising the extracellular antigens secreted into the medium (Specification: page 6, line 15; page 20, lines 19-23; page 35, lines 35-37); and(ii) disrupting the cells in water to provide the intracellular cytoplasmic antigens in a second supernatant which is separated from the disrupted cells (Specification: page 20, lines 24-29; page 35, line 37 through page 36, line 6); and (b) separating the extracellular antigens from the first supernatant (Specification: page 6, lines 10-11).

The invention in Claim 6 is the method of Claim 4 wherein the cells have been disrupted by sonication (Specification: page 20, lines 24-26, page 36, lines 1-2, page 51, lines 2).

The invention in Claim 7 is the method of Claim 4 wherein the *Pythium insidiosum* is deposited as ATCC 74446 (Specification: page 15, lines 28-30, page 20, lines 11-15, page 35, lines 30-35).

The invention in Claim 8 is the method of any one of Claims 5, 6, or 7 wherein the culture medium is Sabouraud dextrose broth (Specification: page 20, lines

13-15; page 35, line 33-35; page 50, lines 30-32).

The invention in Claim 9 is the method of Claim 5 wherein the cells are killed with thimersol (Specification: page 20, lines 18-21; page 8, line 22; page 8, lines 35-37).

The invention in Claim 10 is the method of Claim 5 wherein the disrupted cells are separated from the culture medium for the cells by centrifugation (Specification: page 8, lines 23-24; page 9 line 37 through page 10, line 2).

The invention in Claim 11 is the method of Claim 5 wherein the intracellular cytoplasmic antigens in the second supernatant and the extracellular antigens in the first supernatant are mixed to provide a mixture of antigens (Specification: page 8, lines 24 through page 9, line 1; page 10, line 3-6; page 20, line 35 through page 21, line 2; page 51, lines 4-7), precipitating the mixture of antigens with acetone to provide a precipitate (Specification: page 9, lines 2-3; page 10, lines 7-8; page 21, lines 11-18; page 51, lines 7-10), dissolving the precipitate in sterile distilled water to provide a solution of the antigens (Specification: page 9, lines 3-5; page 10, lines 8-10; page 21, lines 19-22; page 51, lines 10-12), and

dialyzing the solution of antigens in sterile distilled water to remove low molecular weight components less than 10,000 MW to provide the vaccine (Specification: page 9, lines 5-8; page 10, lines 10-12; page 21, lines 23-25; page 51, lines 12-14).

The invention in Claim 12 is the method of Claim 4 wherein the mammal after vaccination is monitored for a change in a Th1 response and a Th2 response, wherein an increase in the Th1 response and a decrease in the Th2 indicates the patient has developed the Th1 response to the vaccine (Specification: page 5, lines 29-34; page 6, lines 9-14; page 7, lines 4-10; Example 6).

(6) Issues

(a) Claims 4-12 were rejected under 35 U.S.C. § 103(a) as being unpatentable over Mendoza *et al.* (1992, *J. Clinical Microbiology*, 30/11:2980-2983) in view of Mendoza *et al.* (1992, *Micopathologia*, 119:89-95). In particular, it is stated in the rejection that it would have been obvious for one skilled in the art at the time the invention was made to combine the two supernatants (supernatants containing intracellular cytoplasmic and extracellular proteins) of the two prior art references with a reasonable expectation of success of obtaining a vaccine for the treatment of infection caused by *Pythium insidiosum*.

(7) Grouping of Claims

Claims 4-10 stand and fall together. Claim 11 stands on its own. Claim 12 stands and falls on its own.

In the method of Claim 11 the intracellular cytoplasmic antigens are precipitated with acetone to provide a precipitate, then dissolved in sterile distilled water and dialyzed in sterile distilled water to remove low molecular weight components less than 10,000 MW to provide the vaccine. The method is distinguishable from the methods in Claims 4-10 and 12 because of the step of dialyzing in sterile distilled water to remove low molecular weight components less than 10,000 MW to provide the vaccine.

In the method of Claim 12 the mammal after vaccination is monitored for a change in a Th1 response and a Th2 response, wherein an increase in the Th1 response and a decrease in the Th2 indicates a Th1 response to the vaccine. The method is distinguishable from the methods in Claims 4-10 and 11 because of this step of monitoring for a change in a Th1 response and a Th2 response.

(8) Argument

Infections caused by fungal and parafungal organisms are occurring with increasing frequency in patients with debilitating illnesses such as leukemia and AIDS, as well as those undergoing immunosuppressive therapy. Among these emerging pathogens is the oomycete *Pythium insidiosum*, a fungal-like organism in the Kingdom Kromista, Phylum Pseudofungi. In lower animals, infections of the cutaneous tissues, lymphatic vessels, intestines, lungs, and bones have been found. In humans, a deadly arteritis infection, subcutaneous invasion, and keratitis occurs. The drugs currently available to treat fungal infections have had little or no effect on *Pythium insidiosum*. Reports of treatment with either amphotericin B or surgery, which are commonly used to treat this disease in humans and lower animals, have indicated that 60% of the patients died of their infections. In cases of arterial invasion in humans, amphotericin B did not eliminate the infection (Rinaldi, M.G., *et al.*, Mycol. Obser. 9:7 (1989); and Thianprasit, M., Trop. Dermathol. 4: 1-4 (1990)), whereas in surgery the main problem has been to determine how much of the infected tissues has to be removed. Thus, relapses are common in surgically

treated patients, who must also endure the pain and distress that such an invasive traumatic procedure inflicts on them.

The presently claimed invention is a method for treatment of pythiosis or prophylaxis against pythiosis in a mammal. It is hypothesized that the mechanism by which the vaccine of the present invention provides its immunotherapeutic effect is by down regulating the T helper 2 (Th2) subset and activating the T helper 1 (Th1) subset. In the natural infection, the hyphae produce extracellular antigens which direct the host's immune system to mount a Th2 response which stimulates production of IgE, IgM, and IgG. The IgE triggers the production of eosinophils and mast cells which degranulate over the hyphae thereby protecting them from the immune system. The hyphae can then multiply and produce overwhelming quantities of extracellular antigens which then locks the host's immune response in the Th2 mode. In the absence of the extracellular antigens the host's immune system is switched to and remains in the Th1 mode, and the host's cytotoxic T lymphocytes and macrophages are then able to destroy the *Pythium insidiosum*.

(a). *It would not have been obvious for one skilled in the art to combine intracellular cytoplasmic and extracellular proteins of the two prior art references for a vaccine when the prior art vaccine comprising intracellular proteins was no more effective than a vaccine with extracellular proteins, had a short shelf-life, and caused inflammation.*

Claims 4-12 were rejected under 35 U.S.C. § 103(a) as being unpatentable over Mendoza *et al.* (1992, *J. Clinical Microbiology*, 30/11:2980-2983) in view of Mendoza *et al.* (1992, *Micopathologia*, 119:89-95). In particular, it is stated in the rejection that it would have been obvious for one skilled in the art at the time the invention was made to combine the two supernatants (supernatants containing intracellular cytoplasmic and extracellular proteins) of the two prior art references with a reasonable expectation of success of obtaining a vaccine for the treatment of infection caused by *Pythium insidiosum*.

Mendoza (*J. Clinical Microbiology*) teaches using the preparation in SDS-polyacrylamide gel electrophoresis to identify immunodominant proteins in the preparation such as the 28, 30, and 32 kD proteins.

Mendoza (*J. Clinical Microbiology*) suggests that the 28, 30, and 32 kD proteins may be useful for diagnostic purposes and immunotherapy, but does not disclose a vaccine.

Mendoza (*Micopathologia*) teaches two vaccines, a soluble concentrated antigen vaccine (SCAV) consisting solely of extracellular antigens that are extruded by the cell into the medium, and a cell-mass vaccine (CMV) consisting of both the soluble and insoluble intracellular antigens. Both vaccines were of limited value for treating horses infected greater than 0.5 months but less than 2 months, and neither vaccine was effective for treating horse that had been infected for more than 2 months. Importantly, Mendoza (*Micopathologia*) teaches that the CMV vaccine is undesirable because it has a short shelf-life and it causes a prominent inflammatory response at the site of inoculation. In neither case were the intracellular proteins separated by SDS-PAGE and then combined with the extracellular proteins as in independent Claim 4.

Basic considerations which apply to obviousness rejections are (A) The claimed invention must be considered as a whole; (B) The references must be considered as a whole and must suggest the

desirability and thus the obviousness of making the combination; (C) The references must be viewed without the benefit of impermissible hindsight vision afforded by the claimed invention; and (D) Reasonable expectation of success is the standard with which obviousness is determined. *Hodosh v. Block Drug Co., Inc.*, 786 F.2d 1136, 1143 n.5, 229 USPQ 182, 187 n.5 (Fed. Cir. 1986).

The claimed method for the treatment of pythiosis or prophylaxis against pythiosis in a mammal, which must be considered as a whole, comprises:

providing an injectable vaccine which comprises in a sterile aqueous solution in admixture: (i) intracellular cytoplasmic antigens separated from disrupted cells of *Pythium insidiosum* by SDS-PAGE; and (ii)extracellular antigens secreted into a medium for growing the cells of the *Pythium insidiosum* wherein the mixture comprises 28, 30 and 32 kDa antigens as determined by SDS-PAGE; and (b) vaccinating the mammal with the vaccine. The references must be considered as a whole and must suggest the desirability and thus the obviousness of making the combination, however there is no suggestion of the desirability of making the combination in the prior art references without the benefit of impermissible hindsight vision afforded by

the claimed invention.

M.P.E.P. § 706.02(j) sets forth the criteria that must shown to establish that a claimed invention is *prima facie* obvious in view of a combination of prior art references. To establish *prima facie* obviousness, it must be shown that (1) there is some suggestion or motivation, either in the prior art references or the general knowledge of one of ordinary skill in the art to combine the reference teachings, (2) there is a reasonable expectation of success if the teachings of the prior art references were combined, and (3) the combined prior art references must teach or suggest all of the claim limitations. It is particularly important to show that there is some reason why one of ordinary skill in the art, with no knowledge of the claimed invention, would have selected the particular prior art references and combined them to render the claimed invention obvious. The case law has repeatedly insisted on such a showing (*See* In re Sang Su Lee, 61 USPQ2d 1430, 1433 (Fed. Cir. 2002), for a brief review of the case law).

In the present case, the prior art provides no suggestion or motivation to one of ordinary skill in the art to combine the prior art references to produce and

use a vaccine like the applicant's for treating mammals. The applicant's presently claimed method uses a vaccine that contains soluble intracellular proteins and the extracellular proteins in a mixture. The fact that the CMV vaccine which contains intracellular proteins causes a prominent inflammatory response would provide no motivation or suggestion to a person of ordinary skill in the art to combine the prior art references and make a vaccine with both extracellular and intracellular components. Having knowledge of Mendoza (*Micopathologia*), one skilled in the art would not have been motivated to make a vaccine that consisted of the SCAV *and* intracellular proteins. It would have been particularly unlikely that one skilled in the art would have made the applicant's vaccine because Mendoza (*Micopathologia*) teaches the CMV having intracellular antigens is unstable and causes a prominent inflammatory reaction at the site of inoculation. Additionally, the CMV vaccine in Mendoza (*Micopathologia*) does not apparently have any advantages as a vaccine as compared to the SCAV, so a person of ordinary skill in the art would find no reason to combine the two. Therefore, a person of ordinary skill in the art would not have been motivated to add intracellular proteins to the SCAV

because in view of the prior art Mendoza (*Micopathologia*), the skilled artisan would have expected the vaccine to have a short shelf-life, limited efficacy, and cause a prominent inflammatory reaction at the site of inoculation. Even though Mendoza (*J. Clinical Microbiology*) teaches a composition containing soluble intracellular proteins but which further includes insoluble proteins, there is nothing in Mendoza (*J. Clinical Microbiology*) or Mendoza (*Micopathologia*) which would suggest to one of ordinary skill in the art that adding only the soluble intracellular proteins to the SCAV would produce a high quality vaccine.

The Federal Circuit has consistently held that absent some teaching or suggestion that would support combining the prior art references, obviousness cannot be established by merely combining the teachings of the prior art to make the applicant's invention. *In re Bell*, 26 USPQ2d 1529 (Fed. Cir. 1993), *In re Fine*, 5 USPQ2d 1596 (Fed. Cir. 1988), and *ACS Hospital Systems v. Montefiore Hospital*, 221 USPQ 929 (Fed. Cir. 1984). Thus, it appears that the present rejection is a hindsight reconstruction of the invention from the applicant's own disclosure, which is not permitted. As

stated in *In re Vaeck*, 947 F.2d 448, 20 USPQ2d 1438, 1442 (Fed. Cir. 1991), "[b]oth the suggestion and the reasonable expectation of success must be founded in the prior art, not in the applicant's disclosure." In the present rejection, the suggestion and motivation for combining the soluble extracellular antigens with the soluble intracellular antigens in the process taught by the applicant to make the vaccine of the present invention becomes obvious only in view of the applicant's disclosure. In the absence of the applicant's disclosure, the prior art teaches that the CMV and SCAV are equivalent in efficacy, and that the SCAV is preferred over the CMV because of its longer shelf-life and its lower inflammatory reaction at the site of injection.

Examiner states that it is *prima facie* obvious to combine two compositions each of which is taught by the prior art to be useful for the same purpose in order to form a third composition that is to be used for the very same purpose; the idea of combining them flows logically from their having been individually taught in the prior art. Therefore, claims that require no more than the mixing together of two conventional items or components sets forth *prima facie* obvious

subject matter. *In re Kerkhoven*, 626 F.2d 846, 205 USPQ 1069 (CCPA 1980).

In a more recent case before the Federal Circuit, based upon the prior art and the fact that each of the three components of the composition used in the claimed method were conventionally employed in the art for treating cooling water systems, the Board had held that it would have been *prima facie* obvious, within the meaning of 35 U.S.C. §103 to employ these components in combination for their known functions and to optimize the amount of each additive. *In re Geiger* 815 F.2d 686, 2 USPQ2d 1276 (Fed. Cir. 1987). The Federal Circuit disagreed and held that "at best, in view of these disclosures, one skilled in the art might find it obvious to try various combinations of these known scale and corrosion prevention agents. However, this is not the standard of 35 U.S.C. §103." *In re Geiger* 2 USPQ2d 1276 at 1278.

Even if a *prima facie* case can be established, the *prima facie* case is rebutted by the fact that Mendoza (*Micopathologia*) teaches away from the combination of a vaccine containing extracellular supernatants with vaccines containing intracellular components such as CMV. Mendoza (*Micopathologia*) teaches two different

methods for producing *Pythium insidiosum* vaccines, (1) a cell-mass vaccine (CMV) containing both the soluble intracellular antigens and insoluble intracellular antigens from the disrupted-cell debris of *P. insidiosum*, and (2) a soluble concentrated antigen vaccine (SCAV) containing only the extracellular antigens that are extruded by *P. insidiosum* into the cell culture medium. Both vaccines are effective as immunotherapy vaccines for curing horses that have been infected with *P. insidiosum* for less than 0.5 months (Mendoza (*Micopathologia*): page 92, Table 1). However, these vaccines are of limited efficacy for curing horses that have been infected for greater than 0.5 months but less than 2 months, and neither vaccine is effective for treating horses that have been infected for more than 2 months (Mendoza (*Micopathologia*): page 92, Table 1, and page 93, Tables 3 and 4). Thus, the CMV and SCAV are of similar efficacy. However, Mendoza (*Micopathologia*) teaches that the SCAV is more practical than the CMV because it retains its effectiveness for up to a year of storage after preparation and has a less violent inflammatory reaction at the site of injection than the CMV (Mendoza (*Micopathologia*): page 94, last paragraph).

Therefore, Mendoza (*Micopathologia*) concludes that the SCAV can be used as the vaccine of choice in early cases of infection (Mendoza (*Micopathologia*): abstract; page 89, last sentence). Thus, Mendoza (*Micopathologia*) teaches that the CMV and SCAV are equivalent in efficacy but that the SCAV vaccine is preferred because of its longer shelf-life and its lower inflammatory reaction. Finally, Mendoza (*Micopathologia*) recommends that the components of the SCAV responsible for immunity be determined (Mendoza (*Micopathologia*): sentence spanning pages 92-93). This recommendation implies that the preferred vaccine should contain only those extracellular antigens of the SCAV which are immunodominant. Considering these facts, Mendoza (*Micopathologia*) leads one skilled in the art away from the vaccine of the present invention which contains soluble intracellular antigens and towards a vaccine that consists of one or more soluble extracellular antigens. The teaching of Mendoza (*Micopathologia*) leads one skilled in the art to believe that a vaccine with intracellular proteins has the disadvantages of causing severe inflammation and having a short storage lifetime without any advantages regarding efficacy.

Mendoza (*J. Clinical Microbiology*) teaches

preparing a mixture of intracellular proteins from *Pythiosis insidiosum* for use in Western blots. Mendoza (*J. Clinical Microbiology*) teaches disrupting the cells and then removing the disrupted cell debris by centrifugation to produce a supernatant containing the proteins. A person of ordinary skill in the art will recognize that the reason the disrupted cell debris was removed from the intracellular proteins was to prevent the disrupted cell debris from clogging up the gel well which will prevent the intracellular antigens from entering the gel. As far as one skilled in the art would know considering Mendoza (*Micopathologia*), the composition of Mendoza (*J. Clinical Microbiology*) would still have a short shelf-life and produce a prominent inflammatory response at the site of inoculation. Since these are undesirable characteristics in a vaccine, one of ordinary skill in the art in view of Mendoza (*J. Clinical Microbiology*) and Mendoza (*J. Clinical Microbiology*) teach away from creating a vaccine containing soluble intracellular proteins for treating Pythiosis in mammals.

Mendoza (*J. Clinical Microbiology*) teaches that intracellular preparations of *P. insidiosum* contain

at least 20 antigens which are recognized by antisera from infected horses, and that three of these antigens appear to be immunodominant. Mendoza (*J. Clinical Microbiology*) does not teach a vaccine containing the immunodominant antigens but suggests that the three immunodominant antigens may be candidates for vaccination trials (Mendoza (*J. Clinical Microbiology*): page 2982, column 2, paragraph 3). However, there is no discussion which would lead a person of ordinary skill in the art to believe that the inflammation problem and the storage life problems have been solved.

In contrast to the prior art vaccines, the vaccine of the present invention is more efficacious than either the CMV or SCAV alone. Unexpectedly, a vaccine which contains both cytoplasmic antigens, which included the 32 kDa, 30 kDa, and 28 kDa immunodominant antigents, and extracellular antigens was able to cure horses that have been chronically infected with *P. insidiosum* for greater than 60 days (Specification: page 23, lines 4-11). The vaccine also cured all horses that are acutely infected with *P. insidiosum* (Specification: page 16, lines 12-13). Furthermore, the vaccine had cured a human who had been infected with *P. insidiosum* for over 60 days (Specification: Example 5, pages 28-

33). These unexpected and remarkable properties of the vaccine of the present invention are in distinct contrast to the CMV and SCAV of the prior art which are only effective against *P. insidiosum* in horses infected for less than 15 days and marginally effective in horses infected for more than 45 days.

It is mere speculation that a vaccine which was prepared by admixing the soluble antigens of the SCAV with the intracellular antigens would provide a vaccine with the same remarkable properties as the vaccine of the present invention. Thus, the vaccine of the present invention is not a merely an admixture of SCAV and intracellular antigens prepared according to the prior art. It would have been unexpected and unobvious that isolating the soluble intracellular antigens from the insoluble intracellular antigens (antigens associated with the cell debris) and then admixing the soluble intracellular antigens with the soluble extracellular antigens extruded into the medium would produce a vaccine with the unexpected and remarkable properties of the vaccine of the present invention, i.e., curing chronically infected horses and humans infected with *Pythiosis*.

Therefore, in light of the above, Claims 4-12

are not obvious over the prior art. Reversal of the rejection and remand to the Examiner for allowance is requested.

Claim 11 is independently patentable since it has the further limitations of precipitating the mixture of antigens with acetone to provide a precipitate, dissolving the precipitate in sterile distilled water to provide a solution of the antigens, and dialyzing the solution of antigens in sterile distilled water to remove low molecular weight components less than 10,000 MW to provide the vaccine.

Mendoza (*J. Clinical Microbiology*) teaches removing the cell debris from the intracellular protein preparation by centrifugation. However, Mendoza (*J. Clinical Microbiology*) does not teach dialyzing the proteins in water to put the proteins in water and to remove material less than 10,000 MW (Claims 1-7) or precipitating the proteins in acetone to remove material that cannot be precipitated with acetone and concentrate the proteins, resuspending the precipitated proteins in water, and then dialyzing the resuspended proteins in water to remove material less than 10,000 MW.

Concentrating the proteins using a PM-10 stir cell is not the equivalent to dialyzing the proteins to remove material less than 10,000 MW because concentrating the proteins in a stir-cell does not reduce the concentration of material less than 10,000 MW

and does not enable the solvent the proteins are in to be exchanged. In contrast, dialysis effects the removal of material less than 10,000 MW and enables the solvent the proteins are in to be exchanged. The dialysis in the present invention performs two functions: it enables material from the protein mixture less than 10,000 MW to be removed, and it enables the solvent the proteins are initially in to be exchanged for water.

Mendoza (*J. Clinical Microbiology*) teaches using a PM-10 membrane in a stir cell to concentrate extracellular proteins of *Conidiobolus coronatus* (Mendoza (*J. Clinical Microbiology*): page 2981), not the intracellular proteins of *Pythium insidiosum*. As taught in Mendoza (*J. Clinical Microbiology*), the medium that the *Conidiobolus coronatus* had been grown in was filtered to remove the cells and the filtrate was placed in a stir-cell with a PM-10 membrane. The proteins were concentrated under positive pressure, which reduces the volume of the filtrate by forcing liquid containing the proteins through the membrane. However, the stir cell does not remove the medium and replace it with water nor does it remove material less than 10,000 MW. The PM-10 membrane merely prevents material greater than 10,000 MW from being lost as the volume of the filtrate is reduced

during the process of forcing the filtrate through the membrane. Therefore, while the concentration of material greater than 10,000 MW in the filtrate increases as the volume of the filtrate is decreased, the concentration of material in the filtrate less than 10,000 MW and the solvent remains the same. Therefore, after concentrating the filtrate using a stir-cell, the filtrate still contains the same concentration of less than 10,000 MW material and the same solvent.

In contrast to concentrating a sample using a stir-cell, dialysis works under the principle of equilibrium to remove small molecules and exchange solvents. In dialysis, a sample such as the above filtrate is placed in bag consisting of a dialysis membrane, which is then placed in a large volume of a solvent. The membrane allows small molecules to pass freely through the membrane while retaining larger molecules which cannot pass through the dialysis membrane. Dialysis membranes are available with different MW cut-offs. For example, the dialysis membrane used by the applicant allows only molecules less than 10,000 MW to pass through the dialysis membrane. Therefore, when the sample in the dialysis membrane is placed in a large volume of solvent that

does not contain the small molecules in the sample, the small molecules diffuse from the sample into the solvent until equilibrium is reached wherein the concentration of small molecules in the solvent becomes the same as the concentration of small molecules in the sample. Thus, the concentration of small molecules in the sample is reduced relative to the concentration of large molecules. By changing the solvent each time after equilibrium is reached, the concentration of small molecules in the sample can be completely removed or reduced to a negligible level. The same principle enables the solvent of the sample to be exchanged with another solvent. Except in the case where the large volume of solvent contains a high salt concentration, dialysis does not result in the sample becoming concentrated; dialysis merely changes the composition of the sample by removing or introducing small molecules that can pass through the membrane. Therefore, a protein preparation containing low molecular weight material that is dialyzed results in a preparation that is distinguishable from the product that results when the same protein preparation is concentrated using a stir-cell because in the former the concentration of low molecular weight molecules is reduced or removed whereas

in the latter the concentration of low molecular weight material remains the same.

Mendoza (*Micopathologia*) does not use stir-cell filtration to prepare the CMV or the SCAV. However, even if one skilled in the art were motivated to combine both the extracellular proteins prepared as taught in Mendoza (*Micopathologia*) and the intracellular proteins as prepared as taught in Mendoza (*J. Clinical Microbiology*) and then concentrate the proteins as taught in Mendoza (*J. Clinical Microbiology*) for *Conidiobolus coronatus*, the resulting preparation would not have been the same as the protein preparation taught by the applicant. The concentrated sample would still contain material less than 10,000 MW and would still be in the same solvent because the stir-cell concentrator merely reduces the volume of a sample; it does not cause the sample to no longer contain material less than 10,000 MW or exchange the solvent. There is nothing in Mendoza (*J. Clinical Microbiology*) and Mendoza (*Micopathologia*) which would have made it *prima facie* obvious to dialyze the proteins in water to put the proteins in water and remove material less than 10,000 MW (Claims 1-7) or to precipitate the proteins with

acetone to remove material that cannot be precipitated by acetone and concentrate the proteins, resuspend the precipitated proteins in water, and dialyze the resuspended proteins in water to remove all the material less than 10,000 MW. Thus, merely combining the intracellular protein preparation for SDS polyacrylamide gel electrophoresis taught in Mendoza (*J. Clinical Microbiology*) with the extracellular protein-containing SCAV taught in Mendoza (*Micopathologia*), with or without the concentration step taught in Mendoza (*J. Clinical Microbiology*) would not have produced the applicant's vaccine used in the method claimed in Claim 11.

Therefore, in light of the above, Claim 11 is not obvious over the prior art. Reversal of the rejection and remand to the Examiner for allowance is requested.

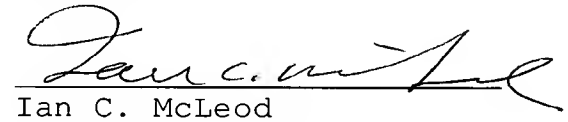
Claim 12 is independently patentable since it has the further limitations wherein the mammal after vaccination is monitored for a change in a Th1 response and a Th2 response, wherein an increase in the Th1 response and a decrease in the Th2 indicates the patient has developed the Th1 response to the vaccine. None of the cited prior art references mention monitoring for a change in the Th1 and a Th2 response, wherein an increase in the Th1 response and a decrease in the Th2 indicates the patient has developed the Th1 response to the vaccine.

Therefore, in light of the above, Claim 12 is not obvious over the prior art. Reversal of the rejection and remand to the Examiner for allowance is requested.

(9) Conclusion

The applicant's vaccine is not obvious in view of Mendoza (*J. Clinical Microbiology*) and Mendoza (*Micopathologia*). For the above reasons it is believed that Claims 4-12, are patentable. Reconsideration of the Examiner's rejection is requested. Allowance of Claims 4-12 is requested.

Respectfully,



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APPENDIX A

-4-

A method for treatment of pythiosis or prophylaxis against pythiosis in a mammal which comprises:

(a) providing an injectable vaccine which comprises in a sterile aqueous solution in admixture:

(i) intracellular cytoplasmic antigens separated from disrupted cells of *Pythium insidiosum* by SDS-PAGE; and

(ii)extracellular antigens secreted into a medium for growing the cells of the *Pythium insidiosum* wherein the mixture comprises 28, 30 and 32 kDa antigens as determined by SDS-PAGE; and

(b) vaccinating the mammal with the vaccine.

-5-

The method of Claim 4 wherein the antigens have been provided by

(a) growing cells of the *Pythium insidiosum* in a culture medium and then

(i) killing the cells;

(ii) separating the killed cells from the culture medium so as to produce a first supernatant comprising the extracellular antigens secreted into the medium; and

(ii) disrupting the cells in water to provide the intracellular cytoplasmic antigens in a second supernatant which is separated from the disrupted cells; and

(b) separating the extracellular antigens from the first supernatant.

-6-

The method of Claim 4 wherein the cells have been disrupted by sonication.

-7-

The method of Claim 4 wherein the *Pythium insidiosum* is deposited as ATCC 74446.

-8-

The method of any one of Claims 5, 6, or 7 wherein the culture medium is Sabouraud dextrose broth.

-9-

The method of Claim 5 wherein the cells are killed with thimersol.

-10-

The method of Claim 5 wherein the disrupted cells are separated from the culture medium for the cells by centrifugation.

-11-

The method of Claim 5 wherein the intracellular cytoplasmic antigens in the second supernatant and the extracellular antigens in the first supernatant are mixed to provide a mixture of antigens, precipitating the mixture of antigens with acetone to provide a precipitate, dissolving the precipitate in sterile distilled water to provide a solution of the antigens, and dialyzing the solution of antigens in sterile distilled water to remove low molecular weight components less than 10,000 MW to provide the vaccine.

-12-

The method of Claim 4 wherein the mammal after vaccination is monitored for a change in a Th1 response and a Th2 response, wherein an increase in the Th1 response and a decrease in the Th2 indicates the patient has developed the Th1 response to the vaccine.